

A New Species of *Yuania* (Orchidaceae) from Southern Nagano, Central Japan

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A new species of *Yuania*, *Yuania flava*, from southern Nagano, central Japan is described. The species has creamy yellow flowers and was formerly identified as *Y. amagiensis* from the Pacific Ocean side of Honshu. *Yuania flava* is morphologically more similar to *Y. japonica* than to *Y. amagiensis*, but is distinguishable from *Y. japonica* by the nearly entire mid-lobe of the lip. DNA sequences from the ITS (internal transcribed spacer) region of the 18S-26S nuclear ribosomal DNA and *rpL* 16 intron further support the hypothesis that *Y. flava* is more akin to *Y. japonica* than to *Y. amagiensis*.

Key words: ITS, orchid, *rpL* 16 intron, southern Nagano, *Yuania flava*

The genus *Yuania* Maxim. (type species *Yuania japonica* Maxim. (Maximowicz 1873)) is circumscribed by such characters as 1) the strongly myco-parasitic habit, 2) the lip with a large slipper-like spur, 3) four hard pollinia with a viscidium, and 4) a unique column structure (cf. Dressler 1993). Until now, five species were described in *Yuania*. One species, *Y. aberrans* Finet from Japan, was transferred to *Cymbidium* Sw. as *Cymbidium aberrans* (Finet) Schltr., and another species, *Y. australis* Hatch, from New Zealand, was moved to *Danhatchia* Garay & Christenson as *Danhatchia australis* (Hatch) Garay & Christenson. *Yuania prainii* King & Pantling from Sikkim has spurless flowers and is unlikely to be closely related to two Japanese species, *Y. japonica* and *Y. amagiensis* Nakai & F. Maek. *Yuania japonica* and *Y. amagiensis* differ in several respects, including flower color, flower posture and rhizome form. Plants of *Yuania* with yellow flowers from southern Nagano Prefecture were previously identified as *Y. amagiensis* of the Pacific Ocean side of Honshu which also has yellowish flowers (Maekawa 1971,

Inoue & Ikegami 1997).

We recognized a few years ago that the plants from southern Nagano differ from *Y. amagiensis* in several points, including flower color and flower posture, and that their patent flowers more closely resemble those of *Y. japonica*. Further, we investigated the DNA sequences to clarify the relationships among the Japanese species of *Yuania*.

Materials and methods

Fresh materials collected for morphological and DNA studies are summarized in Table 1. Based on the results of a global analysis of the tribe Calypsoeae (Yukawa, unpublished) and a systematic treatment by Dressler (1993), *Calypso bulbosa* and *Dactylostalix ringens* were chosen as the outgroup taxa. Fresh materials were preserved in 60 % ethanol for morphological observation. Specimens in the herbaria of Shinshu University (SHIN), National Science Museum, Tokyo (TNS), and the University of Tokyo (TI) were examined.

TABLE 1. Localities of fresh materials collected for use in this study.

taxa	Locality	position collected	voucher for DNA*
<i>Yoania flava</i>	Nagano Pref., Hase V.	whole plant, flower	SHIN 205188
	Nagano Pref., Oshika V.	flower	---
<i>Y. japonica</i>	Nagano Pref. Matsumto C.	flower	---
	Niigata Pref., Myokokogen T.	flower	SHIN 205228
<i>Y. amagiensis</i>	Aomori Pref., Sai V., Arasawa	whole plant, flower	Yukawa 2000-7
	Tokyo Pref., Hachioji C.	whole plant, flower	SHIN 205227
	Saitama Pref., Ogano T.	whole plant	---
<i>Calypso bulbosa</i> var. <i>bulbosa</i>	Aomori Pref., Sai V.	whole plant, flower	Nakajima s.n.
<i>Dactyloctenium aegyptium</i>	Nagano Pref., Fujimi T.	whole plant, flower	Yukawa 2001-3

* SHIN: Herbarium of Shinshu University collection; Yukawa & Nakajima: private collections.

Experimental methods are described in Yukawa *et al.* (1993, 1996). Sequences were determined by amplifying the ITS (internal transcribed spacer) region of the 18S-26S nuclear ribosomal DNA and *rpL* 16 intron of the plastid via the polymerase chain reaction (PCR) from a total DNA extract. We used the primers described in Douzery *et al.* (1999) for ITS and in Jordan *et al.* (1996) for *rpL* 16 intron. In some cases we used the following combination of primers for the amplification and sequencing of *rpL* 16 intron: *rpL*16FIN: 5'-CTTAGTGTGTGACTCGTTAG-3' and *rpL*16R2N: 5'-TGCTTCTATTTGTCTAGCTG-3'. DNA sequences were aligned manually, and gaps were treated as missing characters. The aligned data file is available from the second author upon request. Parsimony and distance analyses were conducted with PAUP* Version 4.0b10 (Swofford 2002). The branch and bound search option was used to perform Fitch parsimony analyses (Fitch 1971). Branch lengths for trees were calculated using the ACC-TRAN optimization (Swofford & Maddison 1987). Distance trees were obtained using the neighbor-joining method (Saitou & Nei 1987) with a Kimura two-parameter correction (Kimura 1980). To assess the relative robustness for branches, the bootstrap method (Felsenstein 1985) was used with 10000 replicates.

Results and Discussion

Description of the new species

Yoania flava K. Inoue et T. Yukawa, **sp. nov.** Figs. 1B, 1D & 2

Yoaniae amagiensis Nakai & F. Maek. et *Y. japonicae* Maxim. similis, sed a priore rhizomatibus repentibus, a posteriore labello et petalis sub-integris, ab ambabus floribus flavis differt.

Type: JAPAN, Honshu, Nagano Pref., Kamiinagun, Hase V, Mae-ura, alt. 1400m, 12 June 2001, K. Inoue 010001 (holo SHIN sh 205588; iso KYO, TI, TNS).

Achlorophyllous geophytes. Plant 10-30 cm tall. Rhizome elongate, creeping, branched repeatedly, internodes 5-10 mm long, ca. 9 mm thick. Stem erect, with several scales near base, loose raceme, 1-8-flowered. Bracts broadly ovate, lower ones 8-12 mm long, much shorter than pedicel and ovary. Pedicel and ovary up to 9 cm long. Flowers creamy yellow, spreading, 3.5-4 cm across. Sepals spreading; dorsal sepal elliptic to oblong, obtuse, 16-19 mm long, 10-11 mm broad; lateral sepals similar in shape and slightly larger. Petals slightly shorter than dorsal sepal, porrect, forming a tube with lip base, elliptic, entire. Lip as long as petals, narrowly ovate, fleshy, obscurely 3-lobed, with conspicuous spur at base; base of lateral lobes with two ridges in front of spur orifice; midlobe of lip ovate, subentire, furrow hairy; spur at base of lip conical, curved anteriorly, 7-10 mm



FIG. 1. A, D: *Yuania flava* (Hase V., Nagano). B, E: *Y. amagiensis* (Hachioji C., Tokyo). C. *Y. japonica* (Myokokogen T., Niigata).

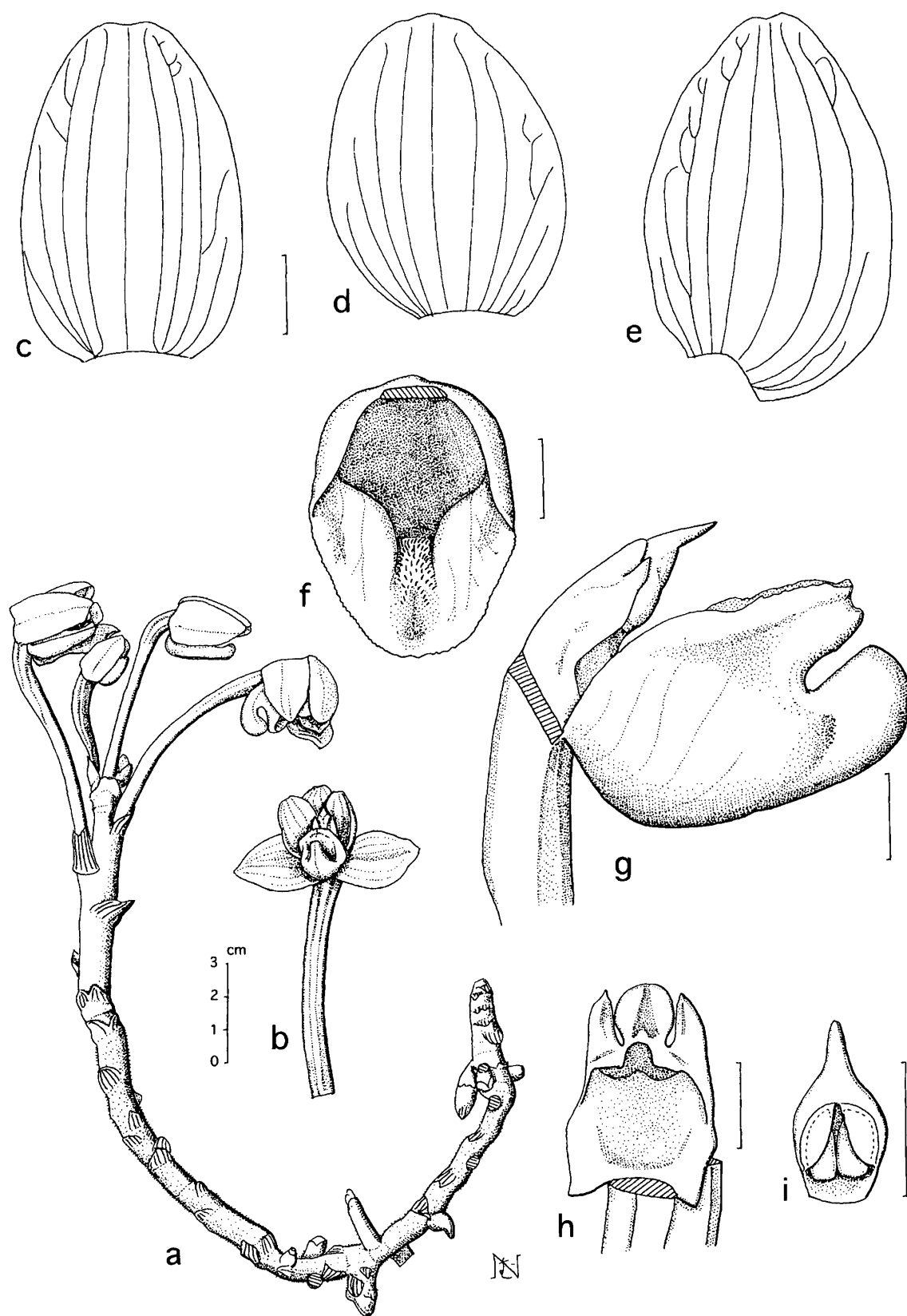


FIG. 2. *Yoania flava*. a, plant; b, flower; c, dorsal sepal; d, petal; e, lateral sepal; f, lip; g, column and lip, side view; h, column (anther removed), ventral view; i, anther, ventral view. a, from Hase V., *K. Inoue 010001*. b-i, from Tatsuno T., *K. Imai s. n.* Scale, 5mm except a and b.

long. Column ovate, ca. 15 mm long, with two distinct obtuse wings; anther ovate-acuminate, with horn, ca. 6 mm long; pollinia 4; stigmas 2, distinct on the hollows under the anther.

Specimens examined: JAPAN. Honshu. Nagano Pref. Suwa Province: Fujimi T., Mt. Shiraiwa (*K. Imai s.n.*, SHIN 177754; *T. Shimizu 25519*, SHIN 15210). Kami-ina Province: Ina C. Katsurakoba (*S. Ohno 105132*, SHIN 72793). Shimo-ina Province: Oshika V., Mt. Toyoguchi (*T. Yamazaki s. n.*, 19 July 1953, TI; *K. Asano 3217*, SHIN 172316; *K. Asano 3868*, TI; *I. Tamaoki & T. Watada s. n.*, 24 May 1964, TI); Iida C., Goshodaira (*K. Asano & T. Yamazaki 10425*, SHIN 172317); Iida C., Warisawa (*K. Asano 3868*, TI; *K. Asano s. n.*, 17 May 1964, TI); Achi V., Misaka Pass (*K. Katsumata 16717-1*, SHIN 172318); Achi V., Mt. Minamizawa (*K. Katsumata 16717-2*, SHIN 172319). Kiso-gun: Narakawa V., Shirakawa (*Y. Matsuda s. n.*, SHIN 153120); Agematsu T., Kurosawa (*H. Okuhara s. n.*, SHIN 146370; *H. Okuhara s. n.*, SHIN 116730); Ohtaki V., Kohrigase (*H. Okuhara s. n.*, SHIN 116761).

Flowering season: mid- June to early July.

Jap. nam.: Shinano-shoki-ran (nov.).

Habitat: Rocky places in broad-leaved deciduous forests. Usually on limestone.

Distribution: Endemic to central Honshu (Nagano Pref.). The distribution of *Y. flava* and *Y. japonica* in Nagano Prefecture is illustrated in Fig. 3. *Yoania flava* occurs mainly in the Ina Province, Nagano Pref.; *Y. japonica* occurs mainly in northern Nagano.

Morphological characters

Yoania flava form Nagano has creeping rhizomes (Fig. 1D & 2a), which differ from the collaroid rhizomes of *Y. amagiensis* (Fig. 1E) and are common with those of *Y. japonica*. Creamy yellow of the flower color (Fig. 1A) differs from yellowish brown of

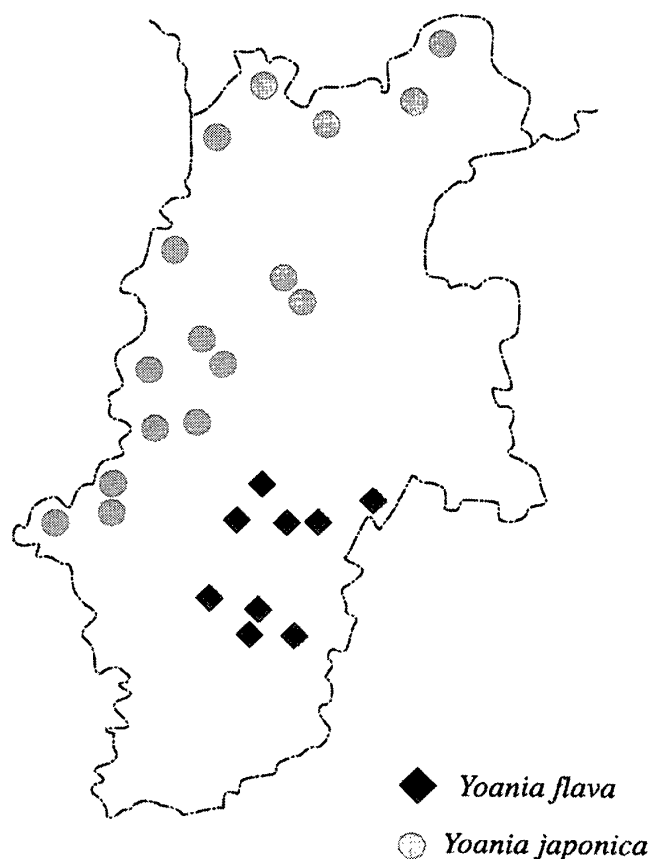


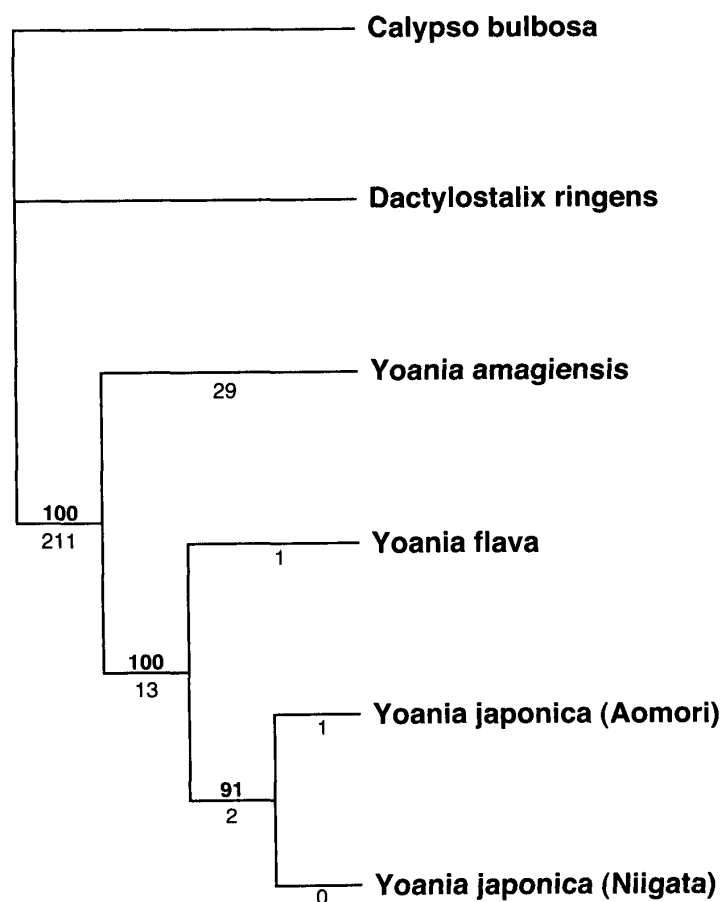
FIG. 3. Distribution of *Yoania flava* and *Y. japonica* in Nagano Pref.

Y. amagiensis (Fig. 1B) and rosy color of *Y. japonica* (Fig. 1C). The southern Nagano plants resemble in flower posture and lax inflorescence to *Y. japonica*. However, the both are different in lip shape. After examining the living materials and herbarium specimens, we concluded that the plants from the southern Nagano represent a new species of *Yoania*, and we described it as *Y. flava*. The comparison of diagnostic characters among *Y. flava*, *Y. japonica* and *Y. amagiensis* is arranged in Table 2.

Key to the Japanese Species of *Yoania*

1. Rhizome elongate, distantly branched; sepals spreading.
 2. Flowers rose-colored, rarely white; midlobe of lip transversely rectangular, erose..... *Y. japonica*
 2. Flowers creamy yellow; midlobe of lip ovate, subentire..... *Y. flava*
1. Rhizome densely branched, collaroid; sepals porrect; flower yellowish brown; midlobe of lip broadly ovate, slightly serrate..... *Y. amagiensis*

	<i>Y. flava</i>	<i>Y. japonica</i>	<i>Y. amagiensis</i>
Rhizome	elongate, creeping	elongate, creeping	densely branched, collaroid
Sepal posture	spreading	spreading	porrect
Lip shape	ovate	ovate, apex truncate	narrowly ovate
Lip margins	almost entire	erose-denticulate	slightly serrate
Petal margins	entire	serrate	slightly serrate
Flower color	creamy yellow	rosy, rarely white	yellowish brown
Inflorescence	laxly 1-6 flowered	laxly 1-7 flowered	densely 5-15 flowered



% divergence from that of *Y. amagiensis*. Within the *rpL16* intron, 11 nucleotide positions were potentially informative out of 1016-1057bp of the determined DNA sequences. This region discriminated each individual by its own autapomorphies. *Yoania flava* and two individuals of *Y. japonica* shared 9

synapomorphies. A single synapomorphy unites the two individuals of *Y. japonica*. Three unambiguous insertions/deletions also link *Y. flava* and two individuals of *Y. japonica* (not included in phylogenetic analyses). A single substitution can be interpreted either as a synapomorphy between the two individuals of *Y. japonica* or between *Y. amagiensis* and *Y. flava* because the character state of the outgroup taxa is inconsistent.

Since the results of the phylogenetic analyses of the ITS data set derived from the nuclear genome information and the *rpL16* data set, which represents plastid lineage, are consistent (results not shown), a combined data set was used for further analyses.

The parsimony analysis resulted in a single most parsimonious tree of 500 steps (Fig.4) and showed a sister group relationship between *Y. flava* and *Y. japonica*. The tree had a consistency index (CI) of 0.988 (0.9688, excluding uninformative characters) and a retention index (RI) of 0.691. The neighbor-joining topology was identical (results not shown). The bootstrap analyses definitely supported the sister group relationship between *Y. flava* and *Y. japonica* (100% support for the parsimony analysis, 89% support for the neighbor-joining analysis). The number of base differences and sequence divergence in the ITS and *rpL16* intron are as follows: *Y. amagiensis*-*Y. flava*: 43, 0.0249; *Y. amagiensis*-*Y. japonica*: 44-45, 0.0253-0.0259; *Y. flava*-*Y. japonica*: 3-4, 0.0017-0.0023; *Y. japonica*-*Y. japonica*: 1, 0.0006. Based on these molecular characters, *Y. flava* is well discriminated from closely related species by its unique base substitutions and most likely has a sister group relationship with *Y. japonica*.

Yoania japonica is widely distributed from Honshu to Taiwan, and westward to Fujian and Jiangxi Provinces, China (Chen *et al.* 1999). *Yoania flava* is restricted to a narrow area of Nagano Prefecture. The distribution and low sequence divergence from *Y. japonica* suggest that speciation of *Y. flava* was a relatively recent event.

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